

Current Smoking, Occupation, *N*-Acetyltransferase-2 and Bladder Cancer: A Pooled Analysis of Genotype-based Studies

Paolo Vineis, Daniela Marinelli, Herman Autrup, Jurgen Brockmoller, Ingolf Cascorbi, Ann K. Daly, Klaus Golka, Henry Okkels, Angela Risch, Nathalien Rothman, Edith Sim, and Emanuela Taioli¹

CPO-Piemonte at the University of Torino, via Santena 7 and Institute for Scientific Interchange Foundation, I-10126 Torino, Italy [P. V.]; Ospedale Policlinico IRCCS and University of Milan, 20122 Milan, Italy [D. M., E. T.]; University of Aarhus, Aarhus C, DK-8000 Denmark [H. A., H. O.]; University of Berlin, Berlin, D-10098 Germany [J. B.]; Ernst Moritz Arndt University, Greifswald, D-17487 Germany [I. C.]; University of Newcastle, Newcastle-upon-Tyne, NE2 4HH United Kingdom [A. K. D.]; Institut für Arbeitsphysiologie, Dortmund, D-44139 Germany [K. G.]; Deutsches Krebsforschungszentrum, Heidelberg, D-69120 Germany [A. R.]; National Cancer Institute, Bethesda, Maryland 20892 [N. R.]; and University of Oxford, Oxford, OX1 3QT United Kingdom [E. S.]

Abstract

The aim of this study was to investigate the association of *NAT2* gene polymorphism with bladder cancer using the data derived from the International Project on Genetic Susceptibility to Environmental Carcinogens. Four case control studies conducted in four European countries, plus two case series, one from England and one from Germany, for a total of 1530 cases and 731 controls (all Caucasian) were included. The interaction between *NAT2* and bladder cancer considering smoking habits and occupational exposure was studied. There was a significant association between *NAT2* and bladder cancer (odds ratio: 1.42, 95% confidence interval: 1.14–1.77), with a slightly significant heterogeneity among studies. However, heterogeneity disappeared when smokers were divided into current and ex-smokers. The risk of cancer was elevated in smokers and occupationally exposed subjects, with the highest risk among slow acetylators. The increase in risk was limited, in fact, to current smokers (odds ratio = 1.74, 95% confidence interval: 0.96–3.15). This analysis confirms that the *NAT2* genotype is a risk factor for bladder cancer by interacting with smoking or occupational exposures. Our observation suggests that *NAT2* is not a risk factor *per se* but modulates the effect of carcinogens contained in tobacco smoke (probably arylamines) or associated with occupational exposures.

Introduction

The *N*-acetyltransferase polymorphism was discovered during the 1950s through the observation of interindividual variability

in the metabolism of isoniazid; such variability was found to be attributable to the presence in about one-half of Caucasian populations of a low activity rate of *N*-acetyltransferase (1). Through the measure of ratios of acetylated to unacetylated metabolites of isoniazide, researchers were able to classify subjects as fast and slow acetylators. Family studies have demonstrated that this metabolic polymorphism depends on variants in the *NAT2* gene, transmitted in an autosomal dominant way. The enzyme has also been shown to polymorphically acetylate arylamines, including well-known bladder carcinogens, to arylamides (2). *N*-acetyltransferase competes in this reaction with *N*-oxidation, which transforms arylamines into active carcinogens. Hence, *N*-acetylation has been interpreted as a detoxifying step in the metabolism of arylamines.

Several studies have been conducted on bladder cancer and the acetylator phenotype. They have been reviewed by Vineis *et al.* (3), Green *et al.* (4), and Johns and Houlston (5), who consistently suggest that slow acetylators have an increased risk of bladder cancer, particularly if they are occupationally exposed to arylamines or smoke cigarettes. In fact, tobacco smoke contains a number of arylamines, including 4-aminobiphenyl (6). Studies of molecular epidemiology have suggested that the excess of bladder cancer in smokers could be attributed to arylamines (7, 8).

More recently, another *N*-acetyltransferase, expressed by the *NAT1* gene, has been found to be polymorphic and to participate in the *N*-acetylation of some carcinogenic aromatic amines, which are also substrates of the *NAT2* enzyme (1, 9).

In an additional meta-analysis of all published case control studies conducted in the general population that had examined the relationship of acetylation status (phenotype and genotype) and bladder cancer risk (22 studies, 2496 cases, and 3340 controls), slow acetylators had a 40% increase in risk compared with rapid acetylators (OR²: 1.4, 95% CI: 1.2–1.6; Ref. 10). However, studies conducted in Asia generated a summary OR of 2.1 (CI: 1.2–3.8), in Europe a summary OR of 1.4 (CI: 1.2–1.6), and in the United States a summary OR of 0.9 (CI: 0.7–1.3). Among European studies, the relationship between *NAT2* slow acetylation and bladder cancer risk did not differ by methods used to assess the acetylation status. In addition, a case series meta-analysis using data from 16 bladder cancer studies conducted in the general population (*n* = 1999 cases) has been published by Marcus *et al.* (11). Because control subjects were unavailable for a number of these studies, the case series design was used. This can be used to assess multiplicative gene-environment interactions; a case series interaction OR > 1.0 indicates that the relationship of cigarette smoking and bladder cancer risk is stronger among slow acetylators as compared with rapid acetylators. The authors observed a weak interaction

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¹ To whom requests for reprints should be addressed, at Ospedale Policlinico IRCCS and University of Milan, Milan, 20122 Italy.

² The abbreviations used are: OR, odds ratio; CI, confidence interval; GSEC, Genetic Susceptibility to Environmental Carcinogens.

Table 1 Studies considered for the pooled analysis (Caucasians only)

Author, year (ref.)	Type of study	Country	No. Cases with NAT2/ No. cases received	No. Published cases with NAT2/No. published cases	No. Controls with NAT2/No. controls received	No. Published controls with NAT2/No. published controls
Brockmoller <i>et al.</i> 1996 (13) ^a	Case control	Germany	427/428	374/374	394/402	373/373 (hospital)
Risch <i>et al.</i> 1995 (14) ^a	Case control	UK	189/189	189/189	43/43	59/59 (hospital)
Peluso <i>et al.</i> 1998, 2000 (15)	Case control	Italy	104/107	114/114	148/163	46/46 (hospital)
Okkels <i>et al.</i> 1997 (16) ^a	Case control	Denmark	254/254	254/254	179/179	242/242 (hospital)
Golka <i>et al.</i> 1997 (17)	Case series	Germany	88/182	88/179	0	0
Daly (unpublished data)	Case control	UK	212/314	Unpublished	Not available	Unpublished
Taylor <i>et al.</i> 1998 (18) ^a	Case control	US (white and black)	Not available	230/230	Not available	203/203 (hospital)
Schnakenberg <i>et al.</i> 1998 (19)	Case control	Germany	Not available	60/60	Not available	154/154 (healthy)

^a Also included in Marcus *et al.* 2000 (Refs. 10 and 11).

between smoking and *N*-acetyltransferase 2 slow acetylation (OR: 1.3, 95% CI: 1.0–1.6) that, again, was stronger when analyses were restricted to studies conducted in Europe (OR: 1.5, 95% CI: 1.1–1.9).

The meta-analyses mentioned above (4, 5, 10, 11) were based on all studies, both phenotype and genotype based. The present analysis is derived from the actual data sets provided by the investigators to the Collaborative Group on GSEC, an initiative that is limited to genotype-based investigations. It includes six studies conducted in Caucasians, all based on genotyping for the *NAT2* gene. The advantage of the present pooled analysis over previous meta-analyses, despite overlapping of the studies that have been included, is the availability of original data sets on confounders and effect modifiers, and in particular, of individual information on smoking habits and occupational exposures.

Materials and Methods

The database collected by the Collaborative Group on GSEC is described elsewhere (12).

Within this database, we have identified all of the genotype-based studies on *NAT2* and bladder cancer. We have included four case control studies conducted in four European countries (13–16), plus two case series, one from England³ and one from Germany (17). Two published studies were not available for the pooled analysis (18, 19). One relevant study in Greece was identified, but the data set was not received in time for the present analysis (20). Studies in non-Caucasians were too limited, and, therefore, we restricted our analyses to Caucasians. All studies were hospital based. Four studies (13, 14, 16, 18) were also included in the papers by Marcus *et al.* (10, 11), which, however, were based on published data and not on the original data sets.

All of the studies gave some information on smoking habits (at least whether ever smoker or nonsmoker); information on the amount smoked and duration were too scanty. Occupational exposures were reported by three studies (13, 14, 16). Cases and controls were interviewed on jobs involving exposure to arylamines. These included jobs in the rubber, textile, chemical, and other industries, in areas with high prevalences of such exposures.

We have computed ORs (Mantel-Haenszel) and the corresponding 95% CIs (21). ORs were adjusted for age, gender, study, and, when relevant, for smoking habits. Additionally, unconditional logistic regression models were fitted. We have

computed the Breslow-Day test for homogeneity of ORs across studies (22) to identify significant heterogeneities. A case series analysis was performed to take advantage of two studies that lacked a control group. This method can be used to assess multiplicative gene environment interaction without inclusion of control subjects.

Results

Table 1 shows the characteristics of the studies available for the present pooled analysis. Four were hospital-based, case control studies, and two were case series. All studies were conducted in Europe, for a total of 1530 cases (415 women and 1115 men) and 731 controls (275 women and 456 men). Table 2 gives the distribution of cases and controls by relevant variables and the corresponding ORs and 95% CIs. As expected, the risk of bladder cancer was greater in men and in older age groups. The association with *NAT2* is expressed by an OR of 1.42 (95% CI: 1.14–1.77), with a slightly significant heterogeneity among studies. The (gender and age adjusted) ORs for the association with *NAT2* in individual case control studies were 1.4 (95% CI: 1.0–1.9; Ref. 13), 2.9 (1.4–5.9; Ref. 14), 1.6 (1.0–2.8; Ref. 15), and 0.9 (0.4–2.1; Ref. 16).

Heterogeneity is evident for the association with smoking habits (OR: 1.69, 95% CI: 1.33–2.14; *P* for homogeneity = 0.001). Heterogeneity is also suggested by the discrepancy between all of the studies (estimate for “ever” versus “never” smokers) and the studies that included information about ex- and current smokers. Heterogeneity is so high that a single overall estimate for smoking is questionable. Finally, the OR for occupational exposures, as defined in the individual studies, was 2.24 (1.70–2.94), with little heterogeneity.

Table 3 shows that the association with *NAT2*, in fact, was absent in nonsmokers, whereas the OR was 1.64 (1.27–2.12) in ever smokers. If we consider the studies that collected the relevant information, the association with *NAT2* was present only in current smokers (OR: 1.74, 95% CI: 0.96–3.15). Heterogeneity was present among “ever smokers.” However, heterogeneity seems to be explained by the inclusion of both current and ex-smokers in the category of ever smokers; when such groups were separated (Table 3), heterogeneity virtually disappeared. The risk of bladder cancer was elevated among the occupationally exposed subjects (Table 3), with the highest risk among slow acetylators who were occupationally exposed.

The case series analysis, performed by pooling cases from all of the six studies (Table 4), confirms a weak interaction between smoking and *NAT2* (26% departure from multiplicativity), which, again, is present only in current smokers (OR: 1.49, 95% CI: 0.84–2.67). None of the ORs in Table 4, how-

³ A. K. Daly, unpublished data.

Table 2 Distribution of bladder cancer cases and controls by *NAT2* genotype, smoking habits, and other characteristics. Caucasians only. ORs adjusted by age, gender, and study (Mantel-Haenszel estimates). 95% CIs (studies: Refs. 13–16)

	Cases	Controls	OR	95% CI
Gender				
Women	306	275	1.0	
Men	722	456	1.33	1.05–1.68
Age <55	90	156	1.0	
55–64	195	205	1.49	1.06–2.09
65–74	359	223	2.53	1.86–3.45
75–79	204	96	3.55	2.42–5.20
80+	179	51	6.16	4.10–9.26
<i>NAT2</i>				
Rapid	327	277	1.0	
Slow	656	402	1.42	1.14–1.77
Breslow-Day test for homogeneity of ORs across studies 7.99 ($P = 0.046$)				
Smoking habits				
Never smokers	219	209	1.0	
Ever smokers	804	513	1.69	1.33–2.14
Known ex-smokers	118	72	2.80	1.70–4.60
Known current smokers	244	204	6.05	3.82–9.57
Breslow-Day test for homogeneity of ORs across studies 47.45 ($P = 0.001$)				
Occupational exposures				
No	400	408	1.0	
Yes	331	130	2.24	1.70–2.94
Breslow-Day test for homogeneity of ORs across studies 1.21 ($P = 0.271$)				

ever, is statistically significant. Individual (gender and age adjusted) ORs for interaction were 1.1 (0.8–1.6) for Brockmoller *et al.* (13), 1.1 (0.5–2.3) for Risch *et al.* (14), 0.9 (0.5–1.5) for Peluso *et al.* (15), 0.7 (0.3–1.5) for Okkels *et al.* (16), 1.7 (0.8–3.4),⁴ and 1.2 (0.3–4.5) for Golka *et al.* (17).

Discussion

The Collaborative Group on GSEC, by collecting and analyzing the epidemiological data sets concerning gene-environment interactions in carcinogenesis, aims at both clarifying open issues and suggesting new hypotheses.

In the present analysis, we have confirmed that the *NAT2* genotype is a risk factor for bladder cancer by interacting with smoking. In fact, the association with bladder cancer was present in smokers only. This observation suggests that *NAT2* is not a risk factor *per se* but modulates the effect of carcinogens contained in tobacco smoke (probably arylamines). Additionally, our observation is clearly consistent with two previous meta-analyses, one based on case control studies that reported an OR of 1.4 (95% CI: 1.2–1.6; Ref. 10) and one among cases only with an OR of 1.3 (1.0–1.6; Ref. 11). Our estimates are almost identical to those reported in the two papers by Marcus *et al.* (10, 11), which considered both phenotype- and genotype-based studies. However, the two analyses are not totally independent, because the present one is based on the original data set of four studies also included in the papers by Marcus *et al.* (10, 11).

Our pooled analysis also suggests, in addition to an interaction between *NAT2* and smoking, that the effect of genetic susceptibility might be present in current smokers only. With the limitations attributable to the low statistical power, the latter observation is interesting, because it suggests that tobacco smoke may exert a late stage action in bladder carcinogenesis, consistent with previous observations (23). In a meta-analysis by Brennan *et al.* (24), a rapid reduction of the risk (about 35%) occurred immediately after cessation of smoking, but the risk remained elevated even after 25 years since quitting, suggesting that some kind of early stage action is also exerted by smoking.

Table 3 Distribution of bladder cancer cases and controls by *NAT2* genotype, smoking habits, and occupational exposures. Caucasians only. ORs from a logistic regression model including gender, age (continuous), and study. 95% CIs (studies: Refs. 13–16).

A. NAT2	Nonsmokers		Ever smokers	
	Cases	Controls	Cases	Controls
Rapid	80	72	242	203
Slow	127	124	523	273
OR (95% CI)	1.07 (0.66–1.75)		1.64 (1.27–2.12)	
Breslow-Day test ^a	1.50 (<i>P</i> = 0.681)		7.92 (0.048)	
Joint effect of smoking and genotype:				
B. NAT2	Nonsmokers		Ever smokers	
Rapid	1.0 (ref.)		1.19 (0.78–1.81)	
Slow	1.07 (0.65–1.75)		1.93 (1.32–2.82)	
C. NAT2	Ex-smokers		Current smokers	
	Cases	Controls	Cases	Controls
Rapid	42	20	57	32
Slow	68	35	157	55
OR (95% CI)	1.00 (0.45–2.07)		1.74 (0.96–3.15)	
Breslow-Day test ^a	1.86 (<i>P</i> = 0.172)		0.96 (0.328)	
Occupational exposures				
D. NAT2	No		Yes	
	Cases	Controls	Cases	Controls
Rapid	127	148	104	48
Slow	250	237	215	68
OR (95% CI) ^b	1.34 (0.96–1.86)		1.50 (0.91–2.45)	
Breslow-Day test ^a	1.67 (<i>P</i> = 0.19)		0.14 (0.70)	
Joint effect of occupational exposure and genotype ^b				
Occupational exposures				
E. NAT2	No		Yes	
Rapid	1.0 (ref.)		2.19 (1.35–3.56)	
Slow	1.24 (0.90–1.71)		3.20 (2.12–4.82)	

^a For homogeneity of ORs across studies.

^b Adjusted for age, gender, study, and smoking habits.

Table 4 Case-only analysis. ORs are adjusted by age, gender, and study (studies: Refs. 13–17, plus Daly, unpublished data)

	NAT2		OR (95% CI)
	Rapid	Slow	
Nonsmokers	107	158	1.0
Smokers	341	671	1.26 (0.91–1.73)
Ex-smokers	105	140	0.99 (0.55–1.79)
Current smokers	93	233	1.49 (0.84–2.67)
Occupational exposure			
No	153	278	1.0
Yes	194	356	0.86 (0.62–1.19)

The hypothesis of a pure late stage action would be at odds with the attribution of a predominant carcinogenic role to arylamines, which are potent mutagens, form electrophilic bonds with DNA, and are expected to be initiating agents. However, a limitation of the present analysis is its being based on hospital controls only. This may hamper the study of the relationship with smoking, because of the potential inclusion in control groups of smoking-related diseases.

Concerning the case series analysis, its validity is conditional on the fact that there is no association between smoking and NAT2 in the general population (or in control series). As a study shows,⁴ there was in fact no relationship between smoking and a number of metabolic polymorphisms in 15,000 controls analyzed in the context of GSEC. Whereas an interaction between occupational exposures and the slow acetylator genotype is suggested in the case control analysis, it is not confirmed in the case-only analysis, possibly because of the lack of statistical power.

A limitation of the present analysis is related to the high degree of heterogeneity observed for the association between smoking and bladder cancer (but not for NAT2), which could be explained by different types of cigarettes smoked in different countries. Heterogeneity was also observed for NAT2 among “ever smokers,” which, however, disappeared when current and ex-smokers were separated.

An additional limitation is the lack of information on other causative/protective factors, in particular, dietary habits, such as the intake of fruit and vegetables, which were available only for a limited number of the studies we have included.

We have not considered the issue of publication bias for two reasons: (a) a formal analysis of publication bias has been considered in previous meta-analyses, which did not suggest this as a plausible explanation of the findings; and (b) the GSEC collaborative initiative has made an effort to approach all of the authors of published and unpublished research, with the limitations indicated above.

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⁴ Gartes *et al.* Metabolic gene polymorphism frequencies in control populations, this issue.